

AMENDMENTS TO THE SPECIFICATION**(1) Sequence Listing**

Please replace the sequence listing paper copy and diskette filed on October 9, 2001, with the substitute sequence listing paper copy and diskette accompanying this submission. See statement under 37 C.F.R. §1.821(f) and (g), in the accompanying sequence submission document.

(2) Title

Please replace the title of the disclosure with the following:

Vectors, Host Cells, and methods for Production of Uridine Phosphorylase and Purine Nucleotide Phosphorylase.

(3) Brief Description of the Drawings

Using the heading "BRIEF DESCRIPTION OF THE DRAWINGS", please insert the following paragraphs into the specification after the paragraph on page 6, line 15, and before the paragraph beginning on page 6, line 16:

Figure 1. Cloning vectors for the expression of UdP and PNP enzymes.

Figures 2A to 2D. 5' and 3' sequences of *upd* and *deoD* genes cloned in plasmid pUC18. Restriction sites of different constructs are underlined. The bases of nucleotide sequences of *upd* and *deoD* genes and the amino acid residues of PNP and UdP proteins are reported in italics. (A) Plasmid pUC18: 5' sequence of *lacZ* gene. (B) Plasmid pGM678 and pGM707: sequence of *lacZ-deoD* fused gene. (C) plasmid pGM679 and pGM708: sequence of *lacZ-upd* fused genes. (D) Plasmid pGM712 and pGM716: 5' and 3' sequence of *deoD* gene.

Figures 3A and 3B. Construction of cloning vectors for the expression of UdP and PNP enzymes.

Figure 4. Construction of cloning vectors for the expression of UdP-(L)-PNP enzymes.

Figure 5. Expression of PNP and UdP in recombinant *E. coli* strains. Gel electrophoresis (SDS-PAGE) of total protein extracts from strains MG1655/pGM707, MG1655/pGM708, and MG1655/pGM716 grown overnight in LD medium supplemented with 12.5 mg/liter of tetracycline. Lanes 15, 2, and 0.3 correspond to protein extracted from 15, 2, and 0.3 ml of bacterial culture.

(4) Disclosure

Please replace the paragraph on page 18, lines 1-15 with the following:

The *E.coli* *udp* gene sequence was found in the EMBL data bank with the accession number X15689. The gene was amplified by PCR with the oligonucleotides 5'-ATCGGTACCATCCATGTCCAAGTCTGATGTTTCATCTC-3' (SEQ ID NO:16) and 5'-AGACGGTCGACAAGAGAATTACAGCAGACGACGC-3' (SEQ ID NO:17) from the *E.coli* strain K12 MG1655 (Singer *et al.*, Microbiol. Rev. 53, 1-24, 1989). The amplified region comprises the entire sequence of the *udp* gene starting from the start codon ATG up to 7 bp downstream of the stop codon TAA. A *Kpn*I restriction site was inserted at the 5' of the gene, followed by four bases selected at random. A *Sal*I site is present at the 3' of the gene. The amplified fragment, digested with *Kpn*I and *Sal*I, was cloned into the polylinker region of the pUC18 vector which carries the ampicillin resistance gene (Yanish and Perron, Gene 33, 103-119, 1985; EMBL accession number L08752). After transformation of the DH5 α strain (Hanahan, J. Mol. Biol. 166, 557-580, 1983), the pGM679 plasmid was obtained (Figure 1). In the construct, a fusion is created between the first codons of the *lacZ* gene of pUC18 and the entire *udp* sequence (Figure 2) and the transcription is under the control of the *lac* promoter of the vector.

Please replace the paragraph on page 18, line 26 to page 19, line 12, with the following:

The *E.coli* *deoD* gene sequence was found in the EMBL data bank with the accession number M60917. The gene was amplified by PCR with the oligonucleotides 5'-

CTGAATTCTTCCATGGCTACCCACACATTAATGCAG-3' (SEQ ID NO:18) and 5'-TCATGGTCGACTTACTCTTATCGCCCAGCAGAACG-3' (SEQ ID NO:19) from the *E.coli* strain K12 MG1655 (Singer *et al.*, Microbiol. Rev. 53, 1-24, 1989). The amplified region comprises the entire sequence of the *deoD* gene starting from the start codon ATG up to the stop codon TAA. An *EcoRI* restriction site was inserted at the 5' of the gene, followed by four bases selected at random. A *SaII* site is present at the 3' of the gene. The amplified fragment, digested with *EcoRI* and *SaII*, was cloned into the polylinker region of the pUC18 vector, which carries the gene for ampicillin resistance (Yanish and Perron, Gene 33, 103-119, 1985; EMBL accession number L08752). After transformation of the DH5 α strain (Hanahan, J. Mol. Biol. 166, 557-580, 1983), the pGM678 plasmid was obtained (Figure 1). In the construct, a fusion is created between the first codons of the *lacZ* gene of pUC18 and the entire *deoD* sequence (Figure 2) and the transcription is under the control of the *lac* promoter of the vector. The cloned region was completely sequenced and was found to be completely identical with the data bank sequence. The pGM678 plasmid sequence is listed.

Please replace the paragraph on page 19, lines 24-30, with the following:

The region containing the tac promoter was obtained by PCR amplification with oligonucleotides 5'-ATTGAGCTCGACATCATAACGGTTCTGGC (SEQ ID NO:20) and 5'-ATTGGATCCTGTGTGAAATTGTTATCCGC (SEQ ID NO:21) of plasmid pGZ119 (Lessl *et al.*, J. Bacteriol. 174, 2493-2500, 1992), digestion of the fragment with *BamHI-SacI* and insertion in *BamHI-SacI* of pGM747 upstream *deoD* gene starting from *tac* promoter and expresses the PNP enzyme identical to the wild-type one. The pGM751 sequence is listed.

Please replace the paragraph on page 20, lines 21-26, with the following:

The fragment *SaII-HindIII*, obtained by PCR amplification using the pGM679 DNA as a template and the oligonucleotides 5'-TCCAGTCGACACAGGAAACAGCTATGA (SEQ ID NO:22) and 5'-TACGAAGCTTA AGAGAATTACAGCAGACG (SEQ ID NO:23), was inserted into plasmid pGM751, digested with *SaII-HindIII*, in order to obtain plasmid pGM800 bearing gene

udp cloned downstream *deoD*. Both genes are transcribed starting from *ptac* but the transduction is independent. The complete sequence of pGM800 is listed.

Please replace the paragraph on page 21, lines 2-16, with the following:

The sequence coding for UdP and PNP have been fused to each other either directly or separated by a short aminoacidic linker. The plasmids were obtained by subsequent steps starting from pGM716. In particular, plasmid pGM716 was digested with *HpaI* and closed again so to have the decision in the terminal part of gene *udp* and in the starting part of *deoD* and create plasmid pGM769 with a unique site *HpaI*. The 3' portion of *udp* was amplified by PCR with the oligonucleotides 5'-GGCCGTTAACCGCACCCAGCAAGAG (SEQ ID NO:24) and 5'-AGCCATGGACAGCAGACGACGCGCC (SEQ ID NO:25); the 5' portion of *deoD* was amplified in the same way with the oligonucleotides 5'-GCTGTCCATGGCTACCCCACACATTAAT (SEQ ID NO:26) and 5'-CCGGGTTAACTTCCAATCGGTGCAGG (SEQ ID NO:27). Subsequently, using the product of the two PCRs as a template and the two extreme sequences, the compete region was amplified: the obtained fragment creates a fusion between *udp* and *deoD*, replacing the *udp* stop codon with a codon for serine, followed by *deoD* ATG codon. The fragment was digested with *HpaI* (site present at the two extremities) and cloned in pGM769 *HpaI* site. The resulting plasmid was called pGM771 (figure 4). In pGM771, the fused protein UdP-PNP is then transcribed starting from *lac* promoter. The plasmid sequence is listed.

Please replace the paragraph on page 21, lines 17-21, with the following:

Plasmid pM771 was subsequently modified by inserting the 5'-CATGGGCGGTGGCAGCCGGGCATTCTGGCCATG (SEQ ID NO:28) linker in the unique *NcoI* site, immediately upstream the starting *deoD* ATG. The resulting plasmid, called pGM795 (figure 4) expresses a fusion protein formed by UdP+ a 11 aminoacid linker (ser-met-gly-gly-gly-ser-pro-gly-ile-leu-ala) (SEQ ID NO:29) + PNP. The pGM795 sequence is listed.